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(54) Title: REGULATION OF DENDRITIC CELL TRAFFICKING

(57) Abstract

Dendritic cells play a critical role in antigen-specific immune responses. Materials and methods are provided for treating disease states, including skin-related and autoimmune disease, by facilitating or inhibiting the migration or activation of dendritic cells. In particular, chemokines are used to initiate, amplify or modulate an immune response.

REGULATION OF DENDRITIC CELL TRAFFICKING

This application is a PCT filing claiming priority to U.S. Patent Application USSN 09/135,215, filed August 17, 1998.

Field of the Invention

The present invention relates generally to the use of mammalian chemokines in the treatment of clinical disease states, including cancer. Administered chemokines direct the migration of dendritic cells, particularly the mature subset. Alternatively, antagonists will block the effects on trafficking at specific locations or compartments.

BACKGROUND OF THE INVENTION

The circulating component of the mammalian circulatory system comprises various cell types, including red and white blood cells of the erythroid and myeloid cell lineages. See, e.g., Rapaport (1987) Introduction to Hematology (2d ed.) Lippincott, Philadelphia, PA; Jandl (1987) Blood: Textbook of Hematology, Little, Brown and Co., Boston, MA.; and Paul (ed. 1993) Fundamental Immunology (3d ed.) Raven Press, N.Y.

Dendritic cells are antigen-presenting cells, and are found in all tissues of the body. They can be classified into various categories, including: interstitial dendritic cells of the heart, kidney, gut, and lung; Langerhans cells in the skin and mucous membranes; interdigitating dendritic cells in the thymic medulla and secondary lymphoid tissue; and blood and lymph dendritic cells. Although dendritic cells in each of these compartments are CD45+ leukocytes that apparently arise from bone marrow, they may exhibit differences that relate to maturation state and microenvironment.

Antigen presentation refers to the cellular events in which a proteinaceous antigen is taken up, processed by antigen presenting cells (APC), and then recognized to initiate an immune response. The most active antigen presenting cells have been characterized as the macrophages (which are direct developmental products from monocytes), dendritic cells, and certain B cells.

For some time, it has been known that the mammalian immune response is based on a series of complex cellular interactions, called the "immune network." Recent research has provided new insights into the inner workings of this network. While it remains clear that much of the response does, in fact, revolve around the network-like interactions of lymphocytes, macrophages, granulocytes, and other cells, immunologists now generally hold the opinion that soluble proteins play a critical role in controlling these cellular interactions. Thus, there is considerable interest in the isolation, characterization, mechanisms of action, and specificity of cell modulatory factors, an understanding of which should lead to significant advancements in the diagnosis and therapy of numerous medical abnormalities, e.g., immune system and other disorders.

The chemokines are a large and diverse superfamily of small soluble proteins. The superfamily is subdivided into two classical branches, based upon whether the first two cysteines in the chemokine motif are adjacent (termed the "C-C" branch), or spaced by an intervening residue ("C-X-C"). A more recently identified branch of chemokines lacks two cysteines in the corresponding motif, and is represented by the chemokines known as lymphotactins. Another recently identified branch has three intervening residues between the two cysteines, e.g., CX3C chemokines. See, e.g., Schall and Bacon (1994) Current Opinion in Immunology 6:865-873; and Bacon and Schall (1996) Int. Arch. Allergy & Immunol. 109:97-109.

Many factors have been identified which influence the differentiation process of precursor cells, or regulate the physiology or migration properties of specific cell types. These observations indicate that other factors exist whose functions in immune function were heretofore unrecognized. These factors provide for biological activities whose spectra of effects may be distinct from known differentiation or activation factors.

However, dendritic cells are poorly characterized, both in terms of responses to soluble factors, and many of their functions and mechanisms of action. Signals regulating the traffic pattern of DC are complex and not fully understood. The absence of knowledge about the physiological properties and responses of these cells limits their understanding. Thus, medical conditions where regulation, development, or physiology of dendritic cells is unusual remain unmanageable.

SUMMARY OF THE INVENTION

The present invention is based, in part, upon the identification of specific chemokines which modulate dendritic cell physiology. In particular, chemokines have been demonstrated to have roles in chemoattracting various cell types.

The present invention provides methods of modulating the attraction, to a chemokine, of a dendritic cell expressing a CCR7 receptor, comprising contacting the cell with a modulating amount of: an agonist ligand for the CCR7 receptor; a mutein antagonist of the ligand for the receptor; an antibody to CCR7 which antagonizes the ligand; or an antibody to the ligand which antagonizes binding of the ligand to the receptor. In certain embodiments, the dendritic cell is a mature dendritic cell; the dendritic cell is an MHC class II^{hi} $B7-2^{hi}$ cell; the ligand is a primate 6Ckine and/or MIP-3β. Often, the the modulating is blocking of the attraction, and the

antibody is a: neutralizing antibody against CCR7; neutralizing antibody against 6Ckine; neutralizing antibody against MIP-3β; or a combination of the neutralizing antibodies. Alternatively, the contacting is with a combination of 6Ckine and MIP-3β. In certain preferred embodiments, the amount is from 30 ng/ml to 300 μg/ml; or the antibody is a monoclonal antibody.

In other preferred embodiments, the dendritic cell is in skin, e.g., wherein the skin is being prepared for a graft, or the skin exhibits the symptoms of a condition selected from the group consisting of: systemic lupus erythematosus; spondyloarthropathies; sclerodermas; acute or chronic inflammation; or atopic or contact dermatitis.

In other embodiments, the MHC class II^{hi} B7-2^{hi} cell is: a bone marrow derived dendritic cell; a langerhans cell; a tissue derived cell; or a lymphoid organ dendritic cell. Perferred embodiments include wherein the modulating is inducing attraction, and the dendritic cell is an MHC class II^{hi}, B7-2^{hi}: cell from skin; or from lymph node. Often, the contacting is with an agonist selected from the group of 6Ckine and MIP-3β.

In other embodiments, the invention provides a mutein variant of a 6Ckine or MIP-3β chemokine comprising a substitution corresponding to a residue in human MIP-3β at position: 19 or 46; 17, 18, 20, or 21; 43, 44, or 45; or the a helical portion of the chemokine structure. In certain embodiments, the substitution is a non-conservative substitution. Others include a sterile composition comprising the variant, and: neutralizing antibody against CCR7; neutralizing antibody against 6Ckine; neutralizing antibody against MIP-3β; or a combination of the neutralizing antibodies.

Additional embodiments include methods of modulating mobility of a dendritic cell, comprising contacting the cell with a modulating amount of: a ligand for the receptor; a mutein antagonist of the ligand for the

receptor; an antibody to CCR7 which antagonizes the ligand; or an antibody to the ligand which antagonizes binding of the ligand to the receptor. In various embodiments, the dendritic cell is a mature dendritic cell; the dendritic cell is an MHC class II^{hi} B7-2^{hi} cell; or the ligand is a primate 6Ckine or MIP-3β; alternatively, the modulating is blocking of the mobility, and the antibody is a: neutralizing antibody against CCR7; neutralizing antibody against 6Ckine; or neutralizing antibody against MIP-3β. Typically, the modulating is inducing attraction, the mature dendritic cell is a class II^{hi}, B7-2^{hi} or resident skin dendritic cell.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

CONTENTS

I.   General

Migration to secondary lymphoid tissues is critical for dendritic cells to present processed antigen to T cells and thereby elicit an immune response. Despite the importance of dendritic cell trafficking in immunity, at present little is understood about the mechanisms of this phenomenon. Here, it is shown that the CCR7 ligands 6Ckine and MIP-3β are potent selective chemoattractants for class II^{hi}, B7-2^{hi} bone marrow-derived dendritic cells, as well as freshly isolated lymph node dendritic cells. Furthermore, these chemokines stimulate the egress of resident skin dendritic cells ex vivo.

Both 6Ckine and MIP-3β exhibit chemoattractant activity for dendritic cells over a broad concentration range (10^{-6} to 10^{-11} M). 6Ckine and MIP-3β are expressed

in lymphoid organs, and 6Ckine has been localized to high endothelial venules and lymphatic endothelium. Based on its expression pattern and potent chemoattractant capability, 6Ckine, in particular, should play an important role in the homing of dendritic cells to lymphoid tissues.

Efficient initiation of T cell dependent immunity is a complex sequence of events that ultimately depends upon simultaneous co-localization of antigen-presenting dendritic cells and antigen-specific $CD4^+$ or $CD8^+$ T cells in the T cell areas of lymphoid organs (Ingulli, et al. (1997) J. Exp. Med. 185:2133-41), leading to activation and expansion of rare T cell clones. Satisfaction of the rather demanding requirements of this rendezvous is facilitated by (1) the constant recirculation of naïve T cells through the lymphoid organs (Butcher and Picker (1996) Science 272:60-66) together with (2) the migration into lymphoid organs of antigen-presenting dendritic cells. Dendritic cells are bone-marrow derived cells that serve a sentinel role in vivo. Steinman (1991) Ann. Rev. Immunol. 9:271-96; and Banchereau and Steinman (1998) Nature 392:245-52).

Immature DCs are distributed throughout many tissues. They are specialized at antigen uptake and processing, but generally express low levels of costimulatory molecules and MHC-peptide complexes on their cell surface. Cella, et al. (1997) Nature 388:782-7; and Pierre, et al. (1997) Nature 388:787-92. Upon stimulation, e.g., by inflammatory signals or CD40 triggering, dendritic cells upregulate costimulatory molecules such as CD86 (B7-2), CD80 (B7-1), and CD40 (Hart (1997) Blood 90:3245-87). Furthermore, their capability to take up antigen is supplanted by a heightened ability to present antigen (Cella, et al. (1997) Nature 388:782-7; and Pierre, et al. (1997) Nature 388:787-92). Activation of dendritic cells ultimately leads to their appearance in T-cell rich areas

of secondary lymphoid organs (Cumberbatch and Kimber (1990) *Immunology* 71:404-10) where, as mature dendritic cells, they interface with recirculating T cells. TCR-mediated recognition of specific antigen presented by these dendritic cells subsequently results in T cell priming. Thus, two parallel homing processes, T cell recirculation through lymphoid tissues and antigen-bearing dendritic cell localization to T cell areas in lymphoid tissues, are largely responsible for efficient immune surveillence.

The ability of dendritic cell to migrate from areas of antigen encounter to sites of T cell priming is fundamental to their capacity to elicit an immune response. However, the mechanisms governing this phenomenon remain largely unknown. The molecular mechanisms of T cell recirculation have been the subject of investigation for several decades, and a number of adhesion molecules participating in this process have been defined. Bargatze, et al. (1995) *Immunity* 3:99-108; Warnock, et al. (1998) *J. Exp. Med.* 187:205-16; Springer (1994) *Cell* 76:301-314.

The role of chemokines, a large family of low molecular weight chemoattractant cytokines, are also being illuminated. Baggiolini (1998) *Nature* 392:565-8. 6Ckine is a recently discovered chemokine that features an unusually long carboxy-terminal tail containing two additional cysteines. Nagira, et al. (1997) *J. Biol. Chem.* 272:19518-24; Hromas, et al. (1997) *J. Immunol.* 159:2554-8; and Hedrick and Zlotnik (1997) *J. Immunol.* 159:1589-93. It is strongly expressed in the T cell zones of lymph nodes, as well as the high endothelial venules (HEV) of lymph nodes and Peyer's patches. Willimann, et al. (1998) *Eur. J. Immunol.* 28:2025-34; and Gunn, et al. (1998) *Proc. Nat'l Acad. Sci. USA* 95:258-63. 6Ckine has been shown to mediate adhesion (Campbell, et al. (1998) *Science* 279:381-4) as well as chemotaxis (see Hedrick and

Zlotnik (1997); Willimann, et al. (1998); Gunn, et al. (1998); and Nagira, et al. (1998) Eur. J. Immunol. 28:1516-23) of T cells, which suggests that it may be important in lymphocyte homing. Interestingly, 6Ckine is also expressed by the endothelial cells lining lymphatic venules (or vessels; Willimann, et al. (1998) and S. Lira) suggesting a role for this chemokine in dendritic cell trafficking to lymph nodes. This distribution pattern suggests that 6Ckine may play an important role in homing of dendritic cells to secondary lymphoid organs.

The present invention is based, in part, upon the observation that 6Ckine, as well as MIP-3β, is an extremely potent inducer of in vitro, as well as in vivo, derived MHC class II^{hi} B7-2^{hi} dendritic cell migration. The ability of 6Ckine and MIP-3β to attract dendritic cells, combined with the limited expression pattern of these chemokines (Nagira, et al. (1997) J. Biol. Chem. 272:19518-24; Hromas, et al. (1997) J. Immunol. 159:2554-8; Hedrick and Zlotnik (1997) J. Immunol. 159:1589-93; Willimann, et al. (1998) Eur. J. Immunol. 28:2025-34; Gunn, et al. (1998) Proc. Nat'l Acad. Sci. USA 95:258-63; Rossi, et al. (1997) J. Immunol. 158:1033-6; and Yoshida, et al. (1997) J. Biol. Chem. 272:13803-9) suggests that they may be key in recruiting dendritic cells into secondary lymphoid organs.

Dendritic cells are key players in both initiation and modulation of the immune response. Central to their role as immune sentinels is their ability to capture, process, and transport antigen to secondary lymphoid tissues where they serve as potent antigen presenting cells capable of stimulating T cells in the T cell area of lymphoid tissues. Trafficking of both T cells and DC to lymphoid organs, followed by precise microenvironmental localization within the T cell area, is critical for efficient immune surveillance and is thought to be directed by chemokines (Baggiolini (1998) Nature 392:565-

8). 6Ckine, a recently discovered CC chemokine (Nagira, et al. (1997) J. Biol. Chem. 272:19518-24; Hromas, et al. (1997) J. Immunol. 159:2554-8; Hedrick and Zlotnik (1997) J. Immunol. 159:1589-93), has been shown to be expressed by the specialized (HEV) in lymph nodes (Gunn, et al. (1998) Proc. Nat'l Acad. Sci. USA 95:258-63), is capable of rapidly triggering integrin binding to vascular ligands (Campbell, et al. (1998) Science 279:381-4), and is a potent chemoattractant for T lymphocytes (Hromas, et al. (1997) J. Immunol. 159:2554-8; Hedrick and Zlotnik (1997) J. Immunol. 159:1589-93; Willimann, et al. (1998) Eur. J. Immunol. 28:2025-34; Gunn, et al. (1998) Proc. Nat'l Acad. Sci. USA 95:258-63; and Nagira, et al. (1998) Eur. J. Immunol. 28:1516-23). These make it a leading candidate for mediating T cell homing.

6Ckine is also expressed by endothelial cells in lymphatic venules (Gunn, et al. (1998) Proc. Nat'l Acad. Sci. USA 95:258-63.), the major route of dendritic cell entry into lymph nodes, further suggesting a role in directing dendritic cell trafficking.

The present disclosure shows that 6Ckine is a potent chemoattractant for bone marrow dendritic cells in vitro and for class II$^+$ CD11c$^+$ skin and lymph node dendritic cells ex vivo. Previous reports (Dieu, et al. (1998) J. Exp. Med. 188:373-86; and Sozzani, et al. (1998) J. Immunol. 161:1083-6) are confirmed that MIP-3β, an alternative CCR7 ligand, is a chemoattractant for dendritic cells in vitro. Furthermore, these findings are extended to show that MIP3β is active on class II$^+$ CD11c$^+$ skin and lymph node dendritic cells ex vivo. The effect of 6Ckine on dendritic cells is far greater than its previously reported ability to chemoattract T cells: 6Ckine (and MIP-3β) attracted dendritic cells at concentrations 1,000 to 10,000 fold lower than those required to attract T cells. Gunn, et al. (1998) Proc.

Nat'l Acad. Sci. USA 95:258-63; and Kim, et al. (1998) J. Immunol. 160:2418-24.

Like many chemokines, 6Ckine binds and signals through multiple receptors; however, 6Ckine is unique in its ability to bind both a CC (CCR7; Yoshida, et al. (1998) J. Biol. Chem. 273:7118-22; and Campbell, et al. (1998) J. Cell Biol. 141:1053-9) and a CXC (CXCR3; Soto, et al. (1998) Proc. Nat'l Acad. Sci. USA 95:8205-10) receptor. The nearly identical response of dendritic cells to the CCR7 ligands 6Ckine and MIP-3β strongly suggested that 6Ckine attracts dendritic cells predominantly via CCR7. PCR analysis of responding bone marrow-derived dendritic cells revealed expression of CCR7, but not CXCR3, supporting the hypothesis that 6Ckine acts through CCR7.

As described above, dendritic cells within the tissues efficiently capture and process antigen and, following activation, upregulate cell surface expression of MHC class II and costimulatory molecules such as B7-2, features associated with mature dendritic cells (Banchereau and Steinman (1998) Nature 392:245-52). These cells then migrate to lymphoid tissues and become potent antigen presenting cells capable of T cell priming. 6Ckine (and MIP-3β) preferentially chemoattracted dendritic cells expressing high levels of MHC class II and B7-2, but were relatively ineffective at attracting MHC class II^{lo} B7-2^{lo} dendritic cells. The selective chemoattraction of dendritic cells displaying a mature phenotype, along with the expression of 6Ckine by lymphatic endothelium, supports a potential role for 6Ckine in mediating migration of mature cells from the tissue into the draining lymph nodes and spleen.

Chemokine responsiveness and chemokine receptor expression have often been studied using dendritic cells generated in vitro from precursors or isolated from blood or other tissue and expanded in vitro. However, chemokine

receptor expression can be achieved by culturing dendritic cells in the presence of cytokines. Delgado, et al. (1998) *Immunobiology* 198:490-500; Dieu, et al. (1998) *J. Exp. Med.* 188:373-86; and Sozzani, et al. (1998) *J. Immunol.* 161:1083-6. Alterations of chemokine receptors on cultured dendritic cells raised the possibility that the findings using bone marrow-derived dendritic cells might not extend to physiologically generated dendritic cells. Nevertheless, experimental evidence is presented that not only in vitro-derived dendritic cells, but also in vivo-derived dendritic cells, can respond to 6Ckine and MIP-3β.

Freshly isolated, uncultured lymph node dendritic cells responded to 6Ckine and MIP-3β in transwell chemotaxis assays. The percentage of lymph node dendritic cells that respond in these assays (about 16% of the input cells) was lower than that observed for cultured bone marrow-derived dendritic cells. However, evidence suggests that the lymph node dendritic cell population is heterogeneous with respect to lineage (Salomon, et al. (1998) *J. Immunol.* 160:708-17; and Steinman, et al. (1997) *Immunol. Rev.* 156:25-37), so that it is possible that 6Ckine may have been active on a particular subset of lymph node dendritic cells, which could not be distinguished in our assays. Further evidence that 6Ckine and MIP-3β could chemoattract in vivo-generated dendritic cells came from observations that incubation of mouse skin in either chemokine increased emigration of dendritic cells out of the skin. Together, these data indicate that dendritic cells obtained from lymphoid as well as nonlymphoid tissues are responsive to CCR7 ligands.

Thus, modulating of chemotaxis or chemokinetic movement will be a statistically significant effect on the movement. Preferably, the effect will be at the half-maximum concentration of the chemokine, but certain effects may be desired at lesser amounts of the chemokine.

Effects may be measured at, e.g., 3x over backgound amounts of chemokine. Thus, the amounts of, or effects of, agonists or antagonists may be more easily detected.

The potential role of 6Ckine and MIP-3β are further suggested by the phenotype of the mutant mouse DDD/1 which, due to an uncharacterized defect in an autosomal recessive gene (*plt* for paucity of lymph node T cells) lacks 6Ckine expression and has dramatically reduced MIP-3β expression in lymph nodes (M.D. Gunn, personal communication). The lymph nodes and Peyer's patches of these mice lack T cells, consistent with observations that while *plt* HEV support lymphocyte rolling, neither *plt* nor wild-type lymphocytes are able to home to lymph nodes when introduced in *plt* mice (Nakano, et al. (1997) Eur. J. Immunol. 27:215-21). Importantly, the *plt* mutation also results in reduced numbers of interdigitating dendritic cells in lymph nodes (M.D. Gunn, personal communication). This observation further supports the hypothesis that CCR7 ligands, and perhaps 6Ckine in particular, influence dendritic cell trafficking to lymph nodes in vivo.

II. Dendritic cells

Dendritic cells (DC) are represented by a diverse population of morphologically similar cell types distributed widely throughout the body in a variety of lymphoid and non-lymphoid tissues. See Caux, et al. (1995) Immunology Today 16:2; and Steinman (1991) Ann. Rev. Immunol. 9:271-296. These cells include lymphoid DC of the spleen, Langerhans cells of the epidermis, and veiled cells in the blood circulation. DC are collectively classified as a group based on their morphology, high levels of surface MHC-class II expression as well as several accessory molecules (B7-1[CD80] and B7-2[CD86]) that mediate T cell binding and costimulation (Inaba, et al. (1990) Intern. Rev. Immunol. 6:197-206; Frendenthal, et al. (1990) Proc. Natl. Acad. Sci. USA

87:769-xxx), and absence of certain other surface markers expressed on T cells, B cells, monocytes, and natural killer cells. DC are specialized antigen-presenting cells which efficiently process and present antigens to, e.g., T cells. They stimulate responses from naive and memory T cells in the paracortical area of secondary lymphoid organs. There is also some evidence for a role in induction of tolerance.

DC are typically bone marrow-derived and migrate as precursors through blood stream to tissues, where they become resident cells such as Langerhans cells in the epidermis. In the periphery, following pathogen invasion, immature DC, e.g., fresh Langerhans cells, are recruited at the site of inflammation (Kaplan, et al. (1992) J. Exp. Med. 175:1717-1728; McWilliam, et al. (1994) J. Exp. Med. 179:1331-1336) where they capture and process antigens (Inaba, et al. (1986) J. Exp. Med. 164:605-613; Streilein, et al. (1989) J. Immunol. 143:3925-3933; Romani, et al. (1989) J. Exp. Med. 169:1169-1178; Puré, et al. (1990) J. Exp. Med. 172:1459-1469; and Schuler, et al. (1985) J. Exp. Med. 161:526-546).

Antigen-loaded DC then migrate from the peripheral tissue via the lymphatic system to the T cell rich area of lymph nodes, where the mature DC are called interdigitating cells (IDC; Austyn, et al. (1988) J. Exp. Med. 167:646-651; Kupiec-Weglinski, et al. (1988) J. Exp. Med. 167:632-645; Larsen, et al. (1990) J. Exp. Med. 172:1483-1494; Fossum (1988) Scand. J. Immunol. 27:97-105; Macatonia, et al. (1987) J. Exp. Med. 166:1654-1667; Kripke, et al. (1990) J. Immunol. 145:2833-2838). At this site, they present the processed antigens to naive T cells and generate an antigen-specific primary T cell response (Liu, et al. (1993) J. Exp. Med. 177:1299-1307; Sornasse, et al. (1992) J. Exp. Med. 175:15-21; Heufler, et al. (1988) J. Exp. Med. 167:700-705). The primary and secondary B-cell follicles contain follicular dendritic

cells that trap and retain intact antigen as immune complexes for long periods of time. These dendritic cells present native antigen to B cells and are likely to be involved being the affinity maturation of antibodies, the generation of immune memory, and the maintenance of humoral immune responses.

III. Chemokines

Known chemokines play an important role in immune and inflammatory responses by inducing migration and adhesion of leukocytes. Oppenheim (1993) Adv. Exp. Med. Biol. 351:183-186; Schall, et al., (1994) Curr. Opin. Immunol. 6:865-873; Rollins (1997) Blood 90:909-928; Baggiolini, et al. (1994) Adv. Immunol. 55:97-179. These small secreted molecules are a growing superfamily of 8-14 kDa proteins originally characterized by a conserved four cysteine motif. See, e.g., Schall (1991) Cytokine 3:165-183; and The Cytokine Handbook Academic Press, NY. Classically, the chemokines are secreted by activated leukocytes, and by stromal cells including endothelial cells and epithelial cells upon inflammatory stimuli (Oppenheim (1993) Adv. Exp. Med. Biol. 351:183-186; Schall, et al. (1994) Curr. Opin. Immunol. 6:865-873; Rollins, (1997) Blood 90:909-928; Baggiolini, et al. (1994) Adv. Immunol. 55:97-179) and act as chemoattractants for a variety of cells which are involved in inflammation. Besides chemoattractant properties, chemokines have been shown to induce other biological responses, e.g., modulation of second messenger levels such as Ca^{++}; inositol phosphate pool changes, see, e.g., Berridge (1993) Nature 361:315-325 or Billah and Anthes (1990) Biochem. J. 269:281-291); cellular morphology modification responses; phosphoinositide lipid turnover; possible antiviral responses; enhancing or suppressing effects on the proliferation of myeloid progenitor cells; and others. Likewise, chemokines may, alone or in combination with

other therapeutic reagents, have similar advantageous combination effects. There are suggestions that chemokines may have effects on other cell types, e.g., attraction or activation of monocytes, dendritic cells, T cells, eosinophils, and/or perhaps on basophils and/or neutrophils. They may also have chemoattractive effects on various neural cells including, e.g., dorsal root ganglia neurons in the peripheral nervous system and/or central nervous system neurons.

The chemokine superfamily was originally divided into two main groups exhibiting characteristic structural motifs, the Cys-X-Cys (C-X-C) and Cys-Cys (C-C) families. These are distinguished on the basis of a single amino acid insertion between the NH-proximal pair of cysteine residues and sequence similarity. Typically, the C-X-C chemokines, e.g., IL-8 and MGSA/Gro-α, act on neutrophils but not on monocytes, whereas the C-C chemokines, e.g., MIP-1α and RANTES, are potent chemoattractants for monocytes and lymphocytes but not neutrophils. See, e.g., Miller, et al. (1992) Crit. Rev. Immunol. 12:17-46. A recently isolated chemokine, lymphotactin, does not belong to either group and may constitute a first member of a third chemokine family, the C family. Lymphotactin does not have a characteristic CC or CXC motif, and acts on lymphocytes but not neutrophils and monocytes. See, e.g., Kelner et al. (1994) Science 266:1395-1399; Schall (1994) "The Chemokines" in The Cytokine Handbook (2d ed.) Academic Press; and Schall and Bacon (1994) Current Opinion in Immunology 6:865-873.

6Ckine and MIP-3β are ligands for CCR7. A comparison of the chemotaxis titrations of human and mouse MIP-3β and mouse 6Ckine on mouse T lymphocytes reveals that human MIP-3β and mouse 6Ckine have very similar chemotaxis curves whereas mouse MIP-3β has a very different curve. Mouse MIP-3β provides chemotactic signals at lower concentrations than human MIP-3β; however, at optimal

concentrations it is a less potent chemoattractant. The titration curve of mouse 6Ckine is very similar to human MIP-3β. Since these chemokines all signal through the same receptor, differences in the amino acid residues of human and mouse MIP-3β may indicate residues important in receptor/ligand binding and receptor signaling. Evaluation of an alignment of the mouse and human sequences of 6Ckine and MIP-3β reveal interesting features. Residue 19 is serine in mouse 6Ckine, tyrosine in human MIP-3β, and asparagine in mouse MIP-3β. Tyrosine and serine have polar, uncharged side chains whereas asparagine has a nonpolar acidic side chain. This residue is predicted to be in the receptor binding face of these molecules, and the dramatic difference in the character of mouse 6Ckine and human MIP-3β vs mouse MIP-3β suggest that this structure may be important in receptor binding. Structural studies of chemokine-receptor interactions suggests that the interaction of chemokine with receptor initially takes place by interaction of the receptor with the amino terminus of the ligand. Thus, significant changes to the amino terminus of the ligand may affect that interaction.

Residue 46 is proline in mouse 6Ckine, arginine in human MIP-3β, and tyrosine in mouse MIP-3β. Proline and arginine have nonpolar side chains and, additionally, arginine has a very basic side chain, whereas tyrosine has an uncharged, polar side chain. This residue is predicted to be in the receptor binding face of these ligand molecules and the dramatic difference in the character of mouse 6Ckine and human MIP-3β vs mouse MIP-3β suggest that this structure may be important in receptor binding. Significant changes, e.g., to aspartic acid, would likely affect agonist effects.

In addition, the structural model of the receptor and ligand interaction suggests that the interaction surface of the chemokine would probably include the residues,

corresponding to mouse MIP3-β: 19 and 46; additionally, residues 17, 18, 20, 21, 43, 44, 45, and the α helical region of the chemokine, e.g., corresponding to WVD... to ...NST near the carboxy terminus. Thus, agonists and antagonists may be developed by appropriate substitution of residues at those positions.

IV. Chemokine effects on dendritic cells

Responses to chemokines are mediated by seven transmembrane spanning G-protein-coupled receptors. Rollins (1997) Blood 90:909-928; Premack, et al. (1996) Nat. Med. 2:1174-1178; Murphy (1994) Ann. Rev. Immunol. 12:593-633. Several chemokines, e.g., monocyte chemotactic protein (MCP)-3, MCP-4, macrophage inflammatory protein (MIP)-1α, MIP-1β, RANTES (regulated on activation, normal T cell expressed and secreted), SDF-1, TECK (thymus expressed chemokine) and MDC (marcrophage derived chemokine), have been reported to attract DC in vitro. Sozzani, et al. (1995) J. Immunol. 155:3292-3295; Sozzani, et al. (1997) J. Immunol. 159:1993-2000; Xu, et al. (1996) J. Leukoc. Biol. 60:365-371; MacPherson, et al. (1995) J. Immunol. 154:1317-1322; Roake, et al. (1995) J. Exp. Med. 181:2237-2247.

While chemokine receptors have been identified on dendritic cells, the effects on them by chemokines have not been well investigated. Chemokines may have been proposed to attract naive dendritic cells to antigen, e.g., tumor associated antigens. However, the role of chemokines in attracting dendritic cells into the secondary immune sites, e.g., lymph nodes, spleen, and collections of mucosa-associated lymphoid tissues, has not been fully established. It is proposed that the means by which skin resident, or other, dendritic cells migrate specifically towards those secondary immune sites, may be mediated by, or supplemented by, chemokines. Those chemokines will be those whose receptors are found on the

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mature, e.g., antigen specific, dendritic cells. The primary chemokines for such are the 6Ckine and MIP-3β.

Conversely, the migration of these dendritic cells to the secondary immune sites might be blocked by antagonists of those same chemokines. Thus, antibody antagonists to the chemokines may find use in preventing further responses. The antagonists may be neutralizing antibodies to the soluble ligands, e.g., 6Ckine or MIP-3β, which can block interaction with the receptors, thereby preventing cellular response. Alternatively, the antagonists may be neutralizing antibodies to the receptor, e.g., CCR7, which block chemokine binding or attraction. Such antagonists should also exhibit the ability to prevent certain chemokinetic movement of the cells.

V.   Therapeutic effects

The present invention provides reagents and methods which will find use in therapeutic applications.

Antibodies and other binding agents directed towards 6Ckine or MIP3β chemokine proteins or nucleic acids will possess significant therapeutic value. The CCR7 chemokine ligands (naturally occurring or recombinant), fragments thereof, and antibodies thereto, along with compounds identified as having binding affinity to a the ligands or receptor, are useful in the treatment of conditions associated with abnormal physiology or development, including immunological reaction or tissue rejection conditions.

Various skin associated medical conditions, e.g., overly active immunological conditions, such as systemic lupus erythematosus, rheumatoid arthritis, spondyloarthropathies, sclerodermas, acute or chronic inflammation, atopic or contact dermatitis, skin grafts or transplants, allergic conditions, or dermatological conditions, may involve the skin or mucosal resident dendritic cells. See, particularly, Frank, et al. (eds.)

Samter's Immunological Diseases Little, Brown, Boston; Kay (ed.) Allergy and Allergic Diseases Blackwell, Oxford; Stone (ed.) Dermatologic Immunology and Allergy Mosby, St. Louis; Norris (ed.) Immune Mechanisms in Cutaneous Disease

5   Dekker, New York; Jordan (ed.) Immunological Diseases of the Skin Appleton and Lange, Norwalk, Conn.; and Beutner, et al. (eds.) Immunopathology of the Skin Wiley, New York; each of which is incorporated herein by reference. Abnormal reactivity or inflammation may be modulated by

10 appropriate therapeutic treatment using the compositions provided herein.   For example, a disease or disorder associated with chemoattractive or chemokinetic signaling by a 6Ckine or MIP-3β chemokine is a target for an agonist or antagonist of the protein.   The proteins likely play a

15 role in regulation or attraction of dendritic cells. Mutein or ligand sequence based antagonists might be created by N-terminal modification, e.g., either truncation of addition of N-terminal extensions, or ligand sequence variants.

20   Other abnormal medical conditions are known to be mediated by abnormal dendritic cell function.   See Reid (1997) Br. J. Haematol. 96:217-223; Steinman (1996) Exp. Hematol. 24:859-862; Berkow (ed.) The Merck Manual of Diagnosis and Therapy, Merck & Co., Rahway, NJ; Holt in

25 Kay (ed.) Allergy and Allergic Diseases Blackwell, Oxford; and Thorn, et al. Harrison's Principles of Internal Medicine, McGraw-Hill, NY.   Developmental or functional abnormalities, e.g., of the hematopoietic or immune system, cause significant medical abnormalities and

30 conditions which may be susceptible to prevention or treatment using compositions provided herein.   Many chemokines have been demonstrated to exhibit chemotactic or chemokinetic activities.

      The distribution of the receptors for these

35 chemokines, especially in dendritic cells or in T cells, along with the ligand expression patterns, suggest roles

in immune functions. Thus, the 6Ckine and MIP-3β chemokines are likely to recruit these cell types in vivo, thereby enhancing the immune response mediated by these cell types. The expression patterns appear consistent
5   with a functional importance of the ligands in initiation of the immune response, particularly in recruiting or mobilizing dendritic cells to the secondary lymph tissues.

Conversely, antagonists will have the opposite effects, and will be useful, e.g., in minimizing
10 autoimmune or suppressing responses in desired contexts, e.g., in a tissue rejection situation. Antagonists may be muteins of the chemokine ligands, antibodies which block binding to receptor, antibodies to the CCR7 receptor, or small drugs which interfere with the ligand-receptor
15 interaction.

Recombinant 6Ckine or MIP-3β chemokine or 6Ckine or MIP-3β chemokine antibodies can be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active or inert
20 ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, e.g., immunogenic adjuvants, along with physiologically innocuous stabilizers and excipients. These combinations can be sterile filtered and placed into dosage forms as by
25 lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof, including forms which are not complement binding.

Alternatively, antibodies to the CCR7 may be used to
30 block signaling by its identified ligands, 6Ckine and MIP-3β. Thus antibody antagonists of 6Ckine and MIP-3β are now available in the form of antibodies to the corresponding receptor.

Drug screening using antibodies or receptor or
35 fragments thereof can identify compounds having binding affinity to 6Ckine or MIP-3β chemokine. Subsequent

biological assays can then be utilized to determine if the compound has intrinsic stimulating activity and is therefore a blocker or antagonist in that it blocks the activity of the protein. This invention further
5   contemplates the therapeutic use of antibodies to 6Ckine or MIP-3β chemokine as antagonists.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state
10   of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents. Animal testing of
15   effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. <u>Goodman and Gilman's: The Pharmacological Bases of Therapeutics</u> (current ed.) Pergamon Press; and <u>Remington's</u>
20   <u>Pharmaceutical Sciences</u> (latest ed.) Mack Publishing Co., Easton, PA. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically
25   acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the <u>Merck Index</u>, Merck & Co., Rahway, NJ. Dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 μM
30   concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 1 fM (femtomolar), with an appropriate carrier. Slow release formulations, or a slow release apparatus will often be utilized for continuous
35   administration.

6Ckine or MIP-3β chemokines, fragments thereof, and antibodies to it or its fragments, antagonists, and agonists, may be administered directly to the host to be treated or, depending on the size of the compounds, it may
5 be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in many conventional dosage formulations, including sterile formulations. While it is possible for the active
10 ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations typically comprise at least one active ingredient, as described above, together with one or more acceptable carriers thereof. Each carrier should be both
15 pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal)
20 administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds. 1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics (8th ed.) Pergamon
25 Press; and (1990) Remington's Pharmaceutical Sciences (17th ed.) Mack Publishing Co., Easton, PA; Avis, et al. (eds. 1993) Pharmaceutical Dosage Forms: Parenteral Medications Dekker, NY; Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Tablets Dekker, NY; and
30 Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, NY. The therapy of this invention may be combined with or used in association with other therapeutic agents, e.g., with other similar reagents directed to the other MIPs, particularly the MIP-
35 1s or MIP-3s.

For example, antagonists can normally be found once the protein has been structurally defined. Testing of potential protein analogs is now possible upon the development of highly automated assay methods using a
5 purified receptor. In particular, new agonists and antagonists will be discovered by using screening techniques described herein.

The broad scope of this invention is best understood with reference to the following examples, which are not
10 intended to limit the inventions in any manner.

EXPERIMENTAL

EXAMPLE 1: General Methods

Some of the standard methods are described or
15 referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.), vols. 1-3, CSH Press, NY; Ausubel, et al., Biology,
20 Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis,
25 centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Coligan, et al. (ed. 1996) and periodic supplements, Current Protocols In Protein Science Greene/Wiley, New York; Deutscher (1990) "Guide to Protein Purification" in
30 Methods in Enzymology, vol. 182, and other volumes in this series; and manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, CA. Combination with recombinant techniques allow fusion to appropriate segments, e.g., to
35 a FLAG sequence or an equivalent which can be fused via a protease-removable sequence. See, e.g., Hochuli (1989)

Chemische Industrie 12:69-70; Hochuli (1990) "Purification
of Recombinant Proteins with Metal Chelate Absorbent" in
Setlow (ed.) Genetic Engineering, Principle and Methods
12:87-98, Plenum Press, N.Y.; and Crowe, et al. (1992)

5   QIAexpress: The High Level Expression & Protein
Purification System QUIAGEN, Inc., Chatsworth, CA.

       Standard   immunological   techniques   are   described,
e.g., in Hertzenberg, et al. (eds. 1996) Weir's Handbook
of Experimental Immunology vols. 1-4, Blackwell Science;

10   Coligan   (1991)   Current   Protocols   in   Immunology
Wiley/Greene, NY; and Methods in Enzymology volumes. 70,
73, 74, 84, 92, 93, 108, 116, 121, 132, 150, 162, and 163.

       Assays for vascular biological activities are well
known   in   the   art.   They   will   cover   angiogenic   and

15   angiostatic activities in tumor, or other tissues, e.g.,
arterial smooth muscle proliferation (see, e.g., Koyoma,
et al. (1996) Cell 87:1069-1078), monocyte adhesion to
vascular epithelium (see McEvoy, et al. (1997) J. Exp.
Med. 185:2069-2077), etc.   See also Ross (1993) Nature

20   362:801-809; Rekhter and Gordon (1995) Am. J. Pathol.
147:668-677;   Thyberg,   et   al.   (1990)   Atherosclerosis
10:966-990; and Gumbiner (1996) Cell 84:345-357.

       Assays for neural cell biological activities are
described, e.g., in Wouterlood (ed. 1995) Neuroscience

25   Protocols modules 10, Elsevier; Methods in Neurosciences
Academic Press; and Neuromethods Humana Press, Totowa, NJ.
Methodology of developmental systems is described, e.g.,
in Meisami (ed.) Handbook of Human Growth and
Developmental Biology CRC Press; and Chrispeels (ed.)

30   Molecular Techniques and Approaches in Developmental
Biology Interscience.

       Computer sequence analysis is performed, e.g., using
available software programs, including those from the GCG
(U. Wisconsin) and GenBank sources.   Public sequence

35   databases were also used, e.g., from GenBank and others.

FACS analyses are described in Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry

5   Methods Wiley-Liss, New York, NY; each of which is which is incorporated herein by reference. Fluorescent activated cell sorting was performed using standard methods on a Becton-Dickinson FACStar PLUS.

10   EXAMPLE 2: PCR analysis.

cDNA libraries prepared from resting bone-marrow derived dendritic cells or bone marrow-derived dendritic cells stimulated overnight with anti-CD40 mAb were subjected to PCR amplification. Plasmid DNA encoding

15   mCCR6, mCCR7, or mCXCR3 served as controls. Appropriate primers used for mCCR6; mCCR7; and mCXCR3. β-actin was amplified as an internal control. PCR products were resolved on a 1.2% agarose gel.

Sequences of chemokines and receptors can be found,

20   e.g., in the GenBank or NCBI databases. See also, R&D Systems, Minneapolis, MN.

EXAMPLE 3: Cells, Antibodies, other Reagents

Bone marrow-derived dendritic cells were generated by

25   culturing bone marrow cell suspensions (Inaba, et al. (1992) J. Exp. Med. 176:1693-702; and Talmor, et al. (1998) Eur. J. Immunol. 28:811-7) obtained from female BALB/c mice (Taconic, Germantown, NY) in RPMI 1640 supplemented with 10% fetal calf serum (FCS), 10 mM Hepes,

30   1 mM sodium pyruvate, 55 μM 2-mercaptoethanol, L-glutamine, penicillin/streptomycin, 10 μg/ml gentamycin sulfate), supplemented with 10 ng/ml GM-CSF and 5 ng/ml IL-4 (Talmor, et al. (1998) Eur. J. Immunol. 28:811-7). Cells were cultured for 5 to 7 days prior to assay.

35   Freshly isolated lymph node cells were prepared by homogenizing lymph nodes from Balb/c mice. The resulting

cell suspension was washed and incubated in RPMI containing 1% FCS for 60 minutes at 37° C prior to assaying. Occasionally, B cells were removed by magnetic depletion, which did not affect the results of chemotaxis

5   assays.

FITC-conjugated anti-I-A^d/I-E^d (2G9) and anti-L-selectin (MEL-14), PE-conjugated anti-CD11c (HL3) and anti-CD45RB (16A), APC-conjugated mAbs directed against B220 (RA3-6B2), CD3 (145-2C11), Gr-1 (RB6-8C5), and CD4

10   (L3T4), and biotinylated anti-B7-2 (GL-1) were from PharMingen (San Diego, CA), as were all relevant isotype control antibodies. Biotinylated anti-B7-2 was detected using streptavidin-CyChrome (PharMingen). Magnetic depletions were performed using anti-CD3 (KT3; Serotec,

15   Kidlington, UK), anti-B220 (RA3-6B2; PharMingen), and anti-Gr-1 (RB6-8C5; kindly provided by B. Coffman, DNAX), followed by incubation with anti-rat immunoglobulin Dynabeads (Dynal, Oslo, Norway). CD40 stimulation of dendritic cells was achieved using mAb 1C10 (Heath, et al.

20   (1994) _Eur. J. Immunol._ 24:1828-34). Murine 6Ckine, MIP-3β, and MIP-1α as well as human MIP-3α and SDF-1α were from R&D Systems (Minneapolis, MN).

EXAMPLE 4: Chemotaxis assays

25   Chemokine proteins are produced, e.g., in COS cells transfected with a plasmid carrying the chemokine cDNA by electroporation. See, Hara, et al. (1992) _EMBO J._ 10:1875-1884. Physical analytical methods may be applied, e.g., CD analysis, to compare tertiary structure to other

30   chemokines to evaluate whether the protein has likely folded into an active conformation. After transfection, a culture supernatant is collected and subjected to bioassays. A mock control, e.g., a plasmid carrying the luciferase cDNA, is used. See, de Wet, et al. (1987) _Mol._

35   _Cell. Biol._ 7:725-757. A positive control, e.g., recombinant murine MIP-1α from R&D Systems (Minneapolis,

MN), is typically used. Likewise, antibodies may be used to block the biological activities, e.g., as a control.

Lymphocyte migration assays are performed as previously described, e.g., in Bacon, et al. (1988) Br. J. Pharmacol. 95:966-974. Other trafficking assays are also available. See, e.g., Quidling-Järbrink, et al. (1995) Eur. J. Immunol. 25:322-327; Koch, et al. (1994) J. Clin. Inv. 93:921-928; and Antony, et al. (1993) J. Immunol. 151:7216-7223. Murine Th2 T cell clones, CDC-25 (see Tony, et al. (1985) J. Exp. Med. 161:223-241) and HDK-1 (see Cherwinski, et al. (1987) J. Exp. Med. 166:1229-1244), made available from R. Coffman and A. O'Garra (DNAX, Palo Alto, CA), respectively, are used as controls.

Ca2+ flux upon chemokine stimulation is measured according to the published procedure described in Bacon, et al. (1995) J. Immunol. 154:3654-3666.

Maximal numbers of migrating cells in response to MIP-1α typically occur at a concentration of 10^{-8} M, in agreement with original reports for CD4+ populations of human T cells. See Schall (1993) J. Exp. Med. 177:1821-1826. A dose-response curve is determined, preferably giving a characteristic bell shaped dose-response curve.

After stimulation with CC chemokines, lymphocytes generally show a measurable intracellular Ca2+ flux. MIP-1α is capable of inducing immediate transients of calcium mobilization. Typically, the levels of chemokine used in these assays will be comparable to those used for the chemotaxis assays (1/1000 dilution of conditioned supernatants).

EXAMPLE 5: Biological activities, direct and indirect

A robust and sensitive assay is selected as described above, e.g., on immune cells, neuronal cells, or stem cells. Chemokine is added to the assay in increasing

doses to see if a dose response is detected. For example, in a proliferation assay, cells are plated out in plates. Appropriate culture medium is provided, and chemokine is added to the cells in varying amounts. Growth is monitored over a period of time which will detect either a direct effect on the cells, or an indirect effect of the chemokine.

Alternatively, an activation assay or attraction assay is used. An appropriate cell type is selected, e.g., hematopoietic cells, myeloid (macrophages, neutrophils, polymorphonuclear cells, etc.) or lymphoid (T cell, B cell, or NK cells), neural cells (neurons, neuroglia, oligodendrocytes, astrocytes, etc.), or stem cells, e.g., progenitor cells which differentiate to other cell types, e.g., gut crypt cells and undifferentiated cell types.

Retroviral infection assays have also been described using, e.g., the CCR1, CCR3, and CCR5 receptors. While the human CCR7 has not been established to be a receptor, the possibility that it might has not been completely eliminated. Recent description of these other chemokine receptors in retroviral infection processes, and the effects by the related RANTES and MIP-1 chemokines, suggest similar effects could exist with the 6Ckine or MIP-3β. See, e.g., Balter (1996) Science 272:1740 (describing evidence for chemokine receptors as coreceptors for HIV); and Deng, et al. (1996) Nature 381:661-666.

Chemokines may also be assayed for activity in hemopoietic assays as described, e.g., by H. Broxmeyer. See Bellido, et al. (1995) J. Clin. Inv. 95:2886-2895; and Jilka, et al. (1995) Expt'l Hem. 23:500-506. They may be assayed for angiogenic activities as described, e.g., by Streiter, et al. (1992) Am. J. Pathol. 141:1279-1284. Or for a role in inflammation. See, e.g., Wakefield, et al. (1996) J. Surgical Res. 64:26-31.

Other assays will include those which have been
demonstrated with other chemokines. See, e.g., Schall and
Bacon (1994) Current Op. Immunol. 6:865-873; and Bacon and
Schall (1996) Int. Arch. Allergy & Immunol. 109:97-109.

5

EXAMPLE 6: Emigration of dendritic cells

Ears from BALB/c mice were aseptically removed and
split into dorsal and ventral halves. Both halves were
cultured in individual wells of a 24 well plate in medium
10 lacking cytokines, in the absence or presence of 10^{-8} to
10^{-7} M 6Ckine or MIP-3β. After 18 to 48 hours of culture
at 37° C, emigrated cells from each ear were stained for
CD11c and I-A^d/I-E^d, and analyzed by FACS. Cells were
quantitated by adding a defined quantity of Dynosphere
15 beads to each sample, and emigrated cell numbers were
corrected for variations in ear weight. The
ultrastructure of emigrated cells was examined in cytospin
preparations.

20 EXAMPLE 7: 6Ckine selectively attracts dendritic cells

As an initial approach to better understand the
chemokines potentially involved in dendritic cell
migration in vivo, the chemotactic response was assessed
of bone marrow-derived dendritic cells in vitro. Bone
25 marrow-derived cells cultured for 5 to 7 days with GM-CSF
and IL-4 were assayed for their ability to migrate across
transwells in response to chemokines. Transmigrated cells
were then identified and enumerated by FACS. This assay
system offers several benefits over other approaches such
30 as Boyden chamber assays, in that 1) large numbers of
cells can be efficiently and objectively quantitated and
2) subsets of cells can be identified without extensive
purification procedures.

Because of these advantages, bone marrow cells were
35 not subjected to any depletion regimen in advance of
assaying chemotactic responses, and the population assayed

contained a mixture of several cell types. Dendritic cells were identified by their distinct morphology in cytospin preparations and by CD11c expression (typically about 25-30% of the total cell population as assessed by
5   FACS), but polymorphonuclear cells expressing B220 and/or Gr-1 (Lin$^+$) were also present.

Cytospin preparations from bone marrow-derived cells cultured in GM-CSF and IL-4 were then tested in transwell chemotaxis assays for their response to 6Ckine. Dendritic
10   cells are greatly enriched in the population. The classII^{hi} B7-2^{hi} subpopulation of dendritic cells is enriched upon migration toward 6Ckine. Bone marrow-derived cells were gated on CD11c$^+$ Lin$^-$ (B220$^-$, Gr-1$^-$) cells and analyzed for their expression of class II and
15   B7-2 before and after chemotaxis in response to 6Ckine. A classII^{hi} B7-2^{hi} and classII^{lo} B7-2^{lo} dendritic cell population is detected; CD11c$^+$ classII$^-$ B7-2$^{lo/-}$ cells are also detected, and likely represent myeloid progenitors. The chemoattractant effect of 6Ckine on classII^{hi} B7-2^{hi}
20   dendritic cells surpasses its effects on classII^{lo} B7-2^{lo} dendritic cells as well as Lin$^+$ cells and naïve CD4$^+$ cells. Bone marrow-derived cells or freshly isolated lymph node cells were tested in parallel in transwell chemotaxis assays in which an increasing concentration of
25   6Ckine was added to the bottom well. The transmigrated populations were stained to identify dendritic cells or Lin$^+$ cells, or naïve CD4$^+$ T cells, and analyzed by FACS.

Among several CC and CXC chemokines assayed, the recently identified CC chemokine 6Ckine (Nagira, et al.
30   (1997) J. Biol. Chem. 272:19518-24; Hromas, et al. (1997) J. Immunol. 159:2554-8; Hedrick and Zlotnik (1997) J. Immunol. 159:1589-93) was able to efficiently and selectively chemoattract a significant proportion of bone marrow-derived cells. The population that responded to 1
35   ng/ml 6Ckine was enriched in dendritic cells, and were 70-75% CD11c$^+$Lin$^-$. The dendritic cells generated from bone

marrow progenitors were heterogeneous for their expression of MHC class II and B7-2, which are upregulated on activated or mature dendritic cells (Steinman (1991) <u>Ann. Rev. Immunol.</u> 9:271-96). Whereas the starting population of $CD11c^+$ Lin^- cells were typically about 25% class II^{hi} $B7-2^{hi}$ and 15% class II^{lo} $B7-2^{lo}$, with the remaining $CD11c^+$ cells likely representing myeloid progenitors, the $CD11c^+$ cells responding to 6Ckine were dramatically enriched for class II^{hi} $B7-2^{hi}$ cells, which were typically 90% or more of the total $CD11c^+$ Lin^- population. 6Ckine was a potent chemoattractant for class II^{hi} $B7-2^{hi}$ dendritic cells over a broad concentration range, routinely attracting 60-90% of this subset of dendritic cells at peak concentrations. In contrast, only a small percentage of class II^{lo} $B7-2^{lo}$ dendritic cells were found in the responding population, and the effective concentration range was much more limited. The apparent specificity of 6Ckine for class II^{hi} $B7-2^{hi}$ dendritic cells was further underscored by the unresponsiveness of contaminating Lin^+ cells except at high concentrations of 6Ckine.

Previous reports describing the chemoattractant abilities of 6Ckine have highlighted its effectiveness in T cell migration. Nagira, et al. (1997) <u>J. Biol. Chem.</u> 272:19518-24; Willimann, et al. (1998) <u>Eur. J. Immunol.</u> 28:2025-34; and Gunn, et al. (1998) <u>Proc. Nat'l Acad. Sci. USA</u> 95:258-63. In agreement with earlier studies, naïve $CD4^+$ T cells responded to 6Ckine in the range of 10^{-6} to 10^{-7} M. It is striking that this concentration was 1,000 to 10,000 times greater than that needed to elicit a response from bone marrow-derived dendritic cells.

Bone marrow-derived dendritic cells exhibit a robust chemotactic response to CCR7 ligands 6Ckine and MIP-3β. Bone marrow-derived cells were tested in transwell chemotaxis assays in which increasing concentrations of individual chemokines were added to the lower well. The

transmigrated populations were stained to identify dendritic cells, and analyzed by FACS.

Several other chemokines have been shown to chemoattract dendritic cells, such as SDF-1α (Sozzani, et al. (1997) J. Immunol. 159:1993-2000; and Delgado, et al. (1998) Immunobiology 198:490-500), MIP-3α (Power, et al. (1997) J. Exp. Med. 186:825-35; and Dieu, et al. (1998) J. Exp. Med. 188:373-86, and MIP-1α (Sozzani, et al. (1997) J. Immunol. 159:1993-2000; Dieu, et al. (1998) J. Exp. Med. 188:373-86; and D'Amico, et al. (1998) Blood 92:207-14), and were therefore compared with 6Ckine in their ability to trigger dendritic cell chemotaxis. In contrast to their response to a wide concentration range of 6Ckine, class II^{hi} B7-2^{hi} dendritic cells only responded to relatively high concentrations of human MIP-3α, and to a limited concentration range of human SDF-1α. Chemotaxis toward murine MIP-1α was weak or absent. Both SDF-1α and MIP-1α were capable of attracting a small percentage of class II^{lo} B7-2^{lo} dendritic cells; the dose-dependence of the response to SDF-1α mirrored that of the class II^{hi} B7-2^{hi} dendritic cells, while MIP-1α was effective at 10 ng/ml (data not shown). Murine MIP-3α and SDF-1α are not currently available, and the low activity of human MIP-3α in particular could be due, at least in part, to species differences.

CD40 ligation leads to upregulation of costimulatory molecules and activation of dendritic cells. Cella, et al. (1996) J. Exp. Med. 184:747-52. In order to determine whether CD40-mediated activation altered dendritic cell responsiveness to 6Ckine, five-day bone marrow-derived cultures were depleted of CD3^{+}, B220^{+}, and most Gr-1^{+} cells and incubated overnight in the presence or absence of anti-CD40 mAb. While CD40 stimulation increased the proportion of dendritic cells that were class II^{hi} B7-2^{hi}, the percentage of class II^{hi} B7-2^{hi} dendritic cells responding to 6Ckine was unchanged. Therefore, it seems

likely that the class II^{hi} B7-2^{hi} dendritic cells present
in unmanipulated cultures represented "mature" dendritic
cells that are phenotypically and functionally (with
respect to chemotaxis) equivalent to CD40-stimulated
5 dendritic cells.

6Ckine shares the chemokine receptor CCR7 with MIP-
3β.   Yoshida, et al. (1997) J. Biol. Chem. 272:13803-9;
and Yoshida, et al. (1998) J. Biol. Chem. 273:7118-22.
Accordingly, the ability of MIP-3β to attract bone marrow-
10 derived dendritic cells was investigated.   Like 6Ckine,
and in contrast to SDF-1α, MIP-3α, and MIP-1α, MIP-3β
attracted dendritic cells over a broad range of
concentrations, and was similar to 6Ckine with respect to
its specificity for class II^{hi} B7-2^{hi} dendritic cells.
15

EXAMPLE 8: Checkerboard Assays
To investigate whether the activity of 6Ckine, MIP-
3β, and the other effective chemokines was chemotactic or
chemokinetic, checkerboard assays were performed.
20 Bone marrow-derived cells were tested in transwell
chemotaxis assays containing various individual chemokines
placed in the upper, lower, or both chambers.   Optimal
concentrations of each chemokine were used as determined
in preliminary titration assays: 6Ckine (6CK) and MIP-3β,
25 10^{-10} M; MIP-3α, 10^{-6} M; and SDF-1α, 10^{-8} M.   Freshly
isolated lymph node cells were assayed in parallel for
their response to 10^{-6} M 6Ckine.   The transmigrated
populations were stained as described to identify
dendritic cells and CD4$^+$ T cells (CD4$^+$ LNC), respectively,
30 and analyzed by FACS.

When optimal concentrations of each chemokine were
added to the upper, lower, or both chambers in transwell
assays, it was repeatedly observed that each chemokine
elicited migration of dendritic cells in a uniform field
35 (i.e., chemokine was present in both upper and lower
chambers), but not in the presence of a negative gradient

(chemokine present only in the upper chamber). However, the number of dendritic cells that migrated through transwells in a uniform field was never equivalent to that achieved in the presence of a positive gradient,

5   suggesting that the response of dendritic cells to all chemokines tested was primarily chemotactic. Similarly, T cells exhibited some degree of chemokinetic activity in the presence of 10^{-6} M 6Ckine.

10   EXAMPLE 9:   Dendritic cells express CCR7

The nearly identical response of dendritic cells to 6Ckine and MIP-3β strongly suggested that both chemokines were exerting their effects through CCR7; however, recent evidence that 6Ckine is also a ligand for CXCR3 (Soto, et

15   al. (1998) <u>Proc. Nat'l Acad. Sci. USA</u> 95:8205-10) raised the possibility that either (or both) CCR7 and CXCR3 may be involved in the observed chemotaxis of dendritic cells in response to 6Ckine. To address this question, the presence of CCR7 and CXCR3 message was assessed in cDNA

20   libraries prepared from bone marrow-derived dendritic cells cultured in the presence or absence of anti-CD40 mAb.

cDNA libraries prepared from bone marrow-derived dendritic cells cultured in the absence or presence of

25   anti-CD40 mAb were subjected to PCR analysis using probes specific for CCR7 or CXCR3. Control reactions were carried out without template or using plasmids expressing the relevant chemokine receptor sequence. β-actin served as an internal control. This analysis revealed that both

30   dendritic cell populations express CCR7, but not CXCR3, suggesting that dendritic cell chemotaxis in response to 6Ckine and MIP-3β is mediated through their common receptor CCR7.

35   EXAMPLE 10:   Lymph node dendritic cells

These results indicated that 6Ckine and MIP-3β are effective chemoattractants for bone marrow-derived dendritic cells cultured in vitro. However, to determine whether these CCR7 ligands were physiologically relevant chemoattractants for dendritic cells, the chemotactic response of freshly isolated lymph node cells to 6Ckine and MIP-3β was assessed in transwell chemotaxis assays.

The response of freshly isolated lymph node cells toward 5 mg/ml of either murine 6Ckine, mMIP-3β, or human MIP-3α was assayed in transwell chemotaxis assays. Dendritic cells in the starting and chemoattracted populations were stained for CD11c, class II, and B7-2, and analyzed by FACS. A small but significant percentage of the class II$^+$ CD11c$^+$ B7-2$^+$ lymph node dendritic cells responded to 6Ckine, peaking at 5 mg/ml (5 x 10^{-6} M) and remaining apparent at concentrations as low as 5 ng/ml (5 x 10^{-10} M). MIP-3β was a consistent, but less potent chemoattractant for lymph node dendritic cells. The differential response to 6Ckine and MIP-3β was also observed when CD4$^+$ T cells were analyzed. There was no response to MIP-3α.

EXAMPLE 11: Resident skin dendritic cells

The ability of in vivo-generated dendritic cells to respond to 6Ckine and MIP-3β was assessed in a second approach, taking advantage of the high density of dendritic cells (Langerhans cells) in skin. Hart (1997) Blood 90:3245-87. Mouse ears were split into dorsal and ventral halves, and cultured overnight in medium containing or lacking either chemokine. Mouse ears were split and cultured overnight in medium lacking cytokines in the absence or presence of 0.1 to 1 mg/ml (10^{-7} to 10^{-6} M) 6Ckine. Emigrated cells were harvested, stained with anti-CD11c and anti-class II mAbs, and analyzed by FACS. Cytospins were prepared from cells obtained from ears cultured overnight in 6Ckine and stained with Giemsa.

6Ckine stimulates the egress of dendritic cells from skin. The emigration of class II^+ $CD11c^+$ cells, which had a distinctly dendritic morphology, was consistently augmented by 6Ckine three- to twelvefold. Similar results

5 were obtained using MIP-3β.

All references cited herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and

10 individually indicated to be incorporated by reference.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way

15 of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

WHAT IS CLAIMED IS:

1.   A method of modulating the attraction to a chemokine of a dendritic cell expressing a CCR7 receptor, comprising contacting said cell with a modulating amount of:

    a) an agonist ligand for said receptor;

    b) a mutein antagonist of said ligand for said receptor;

    c) an antibody to CCR7 which antagonizes said ligand; or

    d) an antibody to said ligand which antagonizes binding of said ligand to said receptor.

2.   The method of Claim 1, wherein:

    a) said dendritic cell is a mature dendritic cell;

    b) said dendritic cell is an MHC class II^{hi} $B7-2^{hi}$ cell; or

    c) said ligand is a primate 6Ckine and/or MIP-3β.

3.   The method of Claim 1, wherein said chemokine is selected from the group of 6Ckine and MIP-3β.

4.   The method of Claim 3, wherein said modulating is blocking of said attraction, and said antibody is a:

    a) neutralizing antibody against CCR7;

    b) neutralizing antibody against 6Ckine;

    c) neutralizing antibody against MIP-3β; or

    d) a combination of said neutralizing antibodies.

5.   The method of Claim 4, wherein said contacting is with a combination of 6Ckine and MIP-3β.

6.   The method of Claim 4, wherein:

    a) said amount is from 30 ng/ml to 300 μg/ml; or

    b) said antibody is a monoclonal antibody.

7.   The method of Claim 1, wherein said dendritic cell is in skin.

8.   The method of Claim 4, wherein said dendritic cell is in skin.

9.   The method of Claim 8, wherein said skin is being prepared for a graft.

10.   The method of Claim 8, wherein said skin exhibits the symptoms of a condition selected from the group consisting of:

  a)   systemic lupus erythematosus;

  b)   spondyloarthropathies;

  c)   sclerodermas;

  d)   acute or chronic inflammation; or

  e)   atopic or contact dermatitis.

11.   The method of Claim 2, wherein said MHC class II^{hi} $B7-2^{hi}$ cell is:

  a)   a bone marrow derived dendritic cell;

  b)   a langerhans cell;

  c)   a tissue derived cell; or

  d)   a lymphoid organ dendritic cell.

12.   The method of Claim 2, wherein said modulating is inducing attraction, and said dendritic cell is an MHC class II^{hi}, $B7-2^{hi}$:

  a)   cell from skin; or

  b)   cell from lymph node.

13.   The method of Claim 12, wherein said contacting is with an agonist selected from the group of 6Ckine and MIP-3β.

14.   A mutein variant of a 6Ckine or MIP-3β chemokine comprising a substitution corresponding to a residue in human MIP-3b at position:

    a)   19 or 46;

    b)   17, 18, 20, or 21;

    c)   43, 44, or 45; or

    d)   the a helical portion of the chemokine structure.

15.   The variant of Claim 14, wherein said substitution is a non-conservative substitution.

16.   A sterile composition comprising said variant of Claim 15, and:

    a)   neutralizing antibody against CCR7;

    b)   neutralizing antibody against 6Ckine;

    c)   neutralizing antibody against MIP-3β; or

    d)   a combination of said neutralizing antibodies.

17.   A method of modulating mobility of a dendritic cell, comprising contacting said cell with a modulating amount of:

    a)   a ligand for said receptor;

    b)   a mutein antagonist of said ligand for said receptor;

    c)   an antibody to CCR7 which antagonizes said ligand; or

    d)   an antibody to said ligand which antagonizes binding of said ligand to said receptor.

18.   The method of Claim 17, wherein:

    a)   said dendritic cell is a mature dendritic cell;

    b)   said dendritic cell is an MHC class II^{hi} B7-2^{hi} cell; or

    c)   said ligand is a primate 6Ckine or MIP-3β.

19.   The method of Claim 17, wherein said modulating is blocking of said mobility, and said antibody is a:
- a) neutralizing antibody against CCR7;
- b) neutralizing antibody against 6Ckine; or
- c) neutralizing antibody against MIP-3β.

20.   The method of Claim 18, wherein said modulating is inducing attraction, said mature dendritic cell is a class II^{hi}, B7-2^{hi} or resident skin dendritic cell.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   A61K38/19   A61K39/395   C07K14/52   //(A61K39/395,38:19)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   C07K   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DIEU M C ET AL: "Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites." JOURNAL OF EXPERIMENTAL MEDICINE, (1998 JUL 20) 188 (2) 373-86. , XP002121899 cited in the application abstract page 373, left-hand column, line 1 -right-hand column, line 9 page 375, left-hand column, paragraph 2 figure 7 page 379, right-hand column, line 3-11 page 378, right-hand column, paragraph 3 -page 379, left-hand column, paragraph 1 page 381, right-hand column, paragraph 3	1-3,7, 11-13, 17,18,20
Y		4-6,16, 19

-/--

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

Date of the actual completion of the international search	Date of mailing of the international search report
8 November 1999	22/11/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Covone, M

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INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 01557 A (SCHERING CORP) 15 January 1998 (1998-01-15) page 5, line 11 -page 6, line 3 page 18, line 5-18 page 25, line 22-32 ---	4-6,16, 19
X	SOZZANI S ET AL: "Differential regulation of chemokine receptors during dendritic cell maturation: a model for their trafficking properties." JOURNAL OF IMMUNOLOGY, (1998 AUG 1) 161 (3) 1083-6. , XP002121900 cited in the application abstract page 1084, left-hand column, paragraphs 3,4 page 1085, right-hand column, paragraph 3 -page 1086, left-hand column, paragraph 1 figure 3 ---	1-3,17, 18,20
A	WILLIMANN K ET AL: "The chemokine SLC is expressed in T cell areas of lymph nodes and mucosal lymphoid tissues and attracts activated T cells via CCR7." EUROPEAN JOURNAL OF IMMUNOLOGY, (1998 JUN) 28 (6) 2025-34. , XP002121901 abstract ---	1-20
A	WO 98 31809 A (IMAI TOSHIO ;SHIONOGI & CO (JP); NAGIRA MORIO (JP); YOSHIE OSAMU ()) 23 July 1998 (1998-07-23) abstract ---	1-20
A	WO 98 26071 A (IMAI TOSHIO ;SHIONOGI & CO (JP); YOSHIDA RYU (JP); YOSHIE OSAMU (J) 18 June 1998 (1998-06-18) abstract ---	1-20
P,X	KELLERMANN S A ET AL: "The CC chemokine receptor-7 ligands 6Ckine and macrophage inflammatory protein-3 beta are potent chemoattractants for in vitro- and in vivo-derived dendritic cells." JOURNAL OF IMMUNOLOGY, (1999 APR 1) 162 (7) 3859-64. , XP002121902 abstract figures 2,5 -----	1-20

2

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 1-6,11-13,17-20 (partially) 7-10 (completely) are
directed to a method of treatment of the human/animal body, the search
has been carried out and based on the alleged effects of the
compound/composition.

Continuation of Box I.1

Claims Nos.: 1-6 9-13 17-20 (partially) 7-8 (completely)

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by
therapy

INTERNATIONAL SEARCH REPORT

Box I   Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

   see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such
   an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

   see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all
   searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report
   covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   [ ] The additional search fees were accompanied by the applicant's protest.

                           [ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-3,7,11,14,17,18 (all partially) 12,13,
        20 (all completely)

    Method of modulating attraction to a chemokine of a dendritic cell expressing a CCR7 receptor comprising contacting said cell with an agonist ligand for said receptor; the methods wherein said agonist is 6Ckine and/or MIP-3beta

2. Claims: 1-3,7,11,14,17,18 (all partially) 4-6,8-10,15,16,
        19 (all completely)

    Method of modulating attraction to a chemokine of a dendritic cell expressing a CCR7 receptor comprising contacting said cell with an antagonist selected from a mutein antagonist ligand for said receptor, neutralizing anti-CCR7 antibodies and anti-6Ckine or anti-MIP-3beta antibodies.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9801557	A	15-01-1998	AU	3574997 A	02-02-1998
			EP	0909321 A	21-04-1999
WO 9831809	A	23-07-1998	AU	5496798 A	07-08-1998
WO 9826071	A	18-06-1998	AU	5410698 A	03-07-1998